Journal of Nematology 26(1):25–31. 1994.

Evaluation of the Spatial Pattern of *Steinernema riobravis* in Corn Plots

H. E. Cabanillas and J. R. Raulston[1]

Abstract: The vertical and horizontal spatial patterns of a naturally occurring population of the entomopathogenic nematode *Steinernema riobravis* (Rhabditida: Steinernematidae) were investigated in corn field soil by laboratory and field bioassays. This nematode appears to be endemic to the Lower Rio Grande Valley of Texas, where it was found parasitizing prepupae and pupae of both corn earworm, *Helicoverpa zea,* and fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Corn earworm prepupa was the bioassay host used to detect *S. riobravis* from soil in previously harvested corn plots. *Steinernema riobravis* occurred at soil depths of 5–30 cm. The maximum nematode density was in the upper 20 cm of soil, and the lowest density occurred at soil depth of 25–30 cm. The field and laboratory bioassays performed on the top 20 cm of soil resulted in *S. riobravis*-infected corn earworm of 49 and 34%, respectively, whereas at 25–30 cm soil depths 11 and 4.5% of the *H. zea* were infected, respectively. The horizontal spatial pattern of this nematode was patchy or aggregated. Our study provides new information on the spatial pattern of *S. riobravis* in its natural habitat and indicates the need to augment its natural biocontrol efficacy.

Key words: biological control, distribution, entomopathogenic nematode, nematode, spatial pattern, *Steinernema riobravis.*

The growing interest in entomopathogenic nematodes is a reflection of their impressive potential for biological control (6, 8,9,10). However, relationships between the occurrence of steinernematid nematodes and the factors regulating nematode performance in the soil have been underinvestigated.

Steinernema riobravis Cabanillas et al., a recently described nematode species, was isolated on 25 July 1990 by Enrique Cabanillas from soil samples in corn fields after harvest in the Lower Rio Grande Valley of Texas where it appears to be indigenous (4). The pathogenicity of *S. riobravis* against corn earworm (CEW), *Helicoverpa* (=*Heliothis*) *zea* (Boddie) has been established (5). Previous observations made in this area and the northeastern part of Tamaulipas, Mexico, indicated that prepupae and pupae of corn earworm and fall armyworm, *Spodoptera frugiperda* (Smith), were naturally infected by *Steinernema* sp. nematodes (11). In the Lower Rio Grande Valley of Texas (15,000 km^2), about 200,000 ha of irrigated corn are planted annually in late February and early March (170,000 ha in Mexico and 30,000 ha in Texas) (12). Generally, fruiting begins in early to mid-May, and mature corn earworm larvae exit the corn to pupate in late May and early June. Little or no pesticide is used to control the CEW and FAW larvae that infest this crop, and *S. riobravis* has good biocontrol potential.

The spatial dispersion of a nematode population is fundamental for its ecological study. Different species of other living organisms have different environmental requirements and behavior patterns (7). Actually, there is no information on the characteristic pattern of spatial dispersion in field soil of *S. riobravis.* More information is needed about steinernematid ecology in their natural soil habitat before they can be used effectively in biocontrol programs (9). The objective of our study was to determine the horizontal and vertical spatial patterns of a naturally occurring population of *S. riobravis* in corn field soil by two bioassay methods.

Materials and Methods

Research site: Bioassays were conducted to determine the spatial pattern of *S. riobravis* in previously harvested corn plots. The field study area was located at the USDA Subtropical Agriculture Research

Received for publication 14 June 1993.

[1] United States Department of Agriculture, Agricultural Research Service, Subtropical Agricultural Research Laboratory, Crop Insects Research Unit, 2413 East Highway 83, Weslaco, TX 78596.

Laboratory South Farm, near Weslaco, Texas. The location was 26° 08.155'N, 97° 57.366'W, and Altitude 21.7 m mean sea level (msl). The hydrometer method (3) was used for soil texture analysis. The soil was a Hidalgo sandy clay loam (47.9% sand, 35.6% clay, 16.5% silt; 1.1% organic matter; pH 8.3; 39.95 meq/100 g Cation exchange capacity [CEC]).

This study area is in the semi-arid subtropics and receives about 600–700 mm of annual rainfall. This field received rain (25 mm) and was irrigated at 5 and 11 days before the field bioassay test, respectively. This site was selected because *S. riobravis* had been observed parasitizing prepupae and pupae of corn earworm and fall armyworm.

Sampling and experimental design: Vertical and horizontal sampling of *S. riobravis* in the soil was done with a systematic-grid sampling design. The experimental design consisted of sixteen 1-m × 1-m plots (1-m quadrants) arranged in four rows each with four plots. Plots were set around the corn plants, and each plot was separated from each other by 20 m within each row and 5 m between rows. Sampling grids were arranged so that one axis of the grid was parallel to the direction of plant rows. Soil samples were taken at six different soil depths (0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm), and each soil depth was replicated 16 times (plots as replicates).

Bioassay host: Corn earworm prepupae were reared in the laboratory and used as a host to detect the presence of *S. riobravis* in the soil in both laboratory and field bioassays. Prepupae were measured (average length = 30 mm, range = 28–32 mm), weighed (average weight = 526 mg, range = 474–545 mg) and stored at 10 C for two days prior to use in the bioassays.

Laboratory bioassay: On 25 October 1990, ten 30-cm deep soil cores were removed from each quadrant with a 5 cm-diam sampling tube in a random pattern with five soil cores coming from each side of the plant row. Each soil sample was divided carefully into 5-cm increments from the surface and combined and mixed by depth. Each pooled depth sample was placed separately in a plastic bag, stored in a styrofoam box, and processed in the laboratory the same day. Soil subsamples were subsequently placed in assay chambers (plastic cups, 7 cm diam, 10 cm height) to detect the presence of nematodes. First, the bottom of the chamber was covered with 2.5 cm of soil from the subsample, followed with 2 corn earworm prepupae, which were then covered with the remaining soil from the subsample (2 corn earworm per depth in 16 plots = 32 corn earworm per depth). The chamber was covered with perforated waxed paper for air exchange and to reduce the rate of evaporation. The chambers were held in a dark room at 23 C for 6 days, after which corn earworms were removed, washed, and individually transferred to "White" trap dishes (15) for an additional 6 days to examine nematode progeny from dead insects. Evaluation of insect mortality was based on the presence of nematode progeny that exited from the cadavers of *H. zea* prepupae and pupae. Soil moisture at each soil depth was determined gravimetrically at the beginning and end of the assay.

Field bioassay: This bioassay was performed in situ on 26 October 1990 using the same plots from which soil samples had been removed for the laboratory bioassay. Within each plot, four 1.5 cm-diam holes were made by pushing an iron rod into the soil to a depth of 30 cm. The holes were spaced about 15 cm apart on each side of the corn plants remaining in the plots. Corn earworm prepupae individually contained in hardware cloth (8 mesh) cages, 5 cm × 1.5 cm diam, were lowered into each hole to soil depths of 5, 10, 15, 20, 25, and 30 cm (4 corn earworm per depth in 16 plots = 64 corn earworm per depth). Original soil for a particular depth was pressed around the cages to maintain soil contact and allow ingress of nematodes through the cages to the host. Five hundred ml of water was added to the soil surface around each set of cages. After 6 days, the cages were taken out of the soil and washed; corn earworms were then individ-

ually transferred to "White" trap dishes (15) for an additional 6 days to examine nematode progeny from dead insects. Insect mortality was evaluated on the presence of nematode progeny that exited from the cadavers of corn earworm prepupae and pupae.

Soil temperature data were obtained through copper-constantan thermocouples located at six soil depths (5, 10, 15, 20, 25, and 30 cm) in the corn field. Thermocouples were connected to a Model 592R General Purpose Interface Board, and the temperature data were logged at 30-minute intervals with an Omnidata, Polycorder® Model 516B (Omnidata International Inc., Logan, UT). Soil moistures at each depth were determined gravimetrically at the beginning and end of the bioassays.

Steinernema riobravis occurrence in the field soil was estimated using the proportion of dead insects infected by *S. riobravis*. Insect mortality was based on nematode progeny at 12 days after insects were exposed to natural field soil. However, for the field bioassay we compared insect mortality at 6 and 12 days after corn earworm were exposed to soil. The first evaluation (6 days) was based on symptoms (*Steinernema*-infected insects are often flaccid and change color to yellow or brown, and their tissues have a gummy consistency). The second evaluation (12 days), was based on the presence of nematode progeny that exited from the cadavers of prepupal and pupal stages of *H. zea*. We did not dissect the insects to determine nematode presence because we were interested in knowing the capacity of *S. riobravis* to develop progeny after insect death. Infective juveniles started exiting from infected hosts about 10 days after insects were exposed to natural soil. Nematodes were collected to verify the identity of *S. riobravis* based on morphological characteristics of the infective juveniles and males (4).

Data analysis: Data comparing the vertical occurrence of *S. riobravis* were analyzed by regression analysis using PROC REG of SAS (13). The analysis was performed on the data collected from dead insects that showed nematode progeny (12 days). Insect mortality (dependent variable) was regressed against soil depth (independent variable) using linear and quadratic models. The coefficient of determination (R^2) and plots of standardized residuals vs. predicted values from regression analysis were used to evaluate goodness of fit to a model.

Data analysis for the horizontal spatial pattern of *S. riobravis* were compared at three soil depths (10, 20, and 30 cm) using the chi-squared, variance-to-mean ratio for agreement with a Poisson series (small samples, $n < 30$) according to Elliot (7). The index of dispersion (I) or variance-to-mean ratio was calculated: $I = s^2/\bar{x}$, where s^2 = sample variance and $\bar{x}$ = sample arithmetic mean. The significance of departures from unity was assessed by reference to a table of chi-square values (7). The index of dispersion (k) for the negative binomial was calculated as follows: $k = \bar{x}^2/(s^2 - \bar{x})$; where: $\bar{x}$ = arithmetic mean, and s^2 = sample variance.

Results

The average soil temperature at different soil depths (5, 10, 15, 20, 25, 30 cm) in the corn plots was 21 C (maximum 25 C, minimum 16 C). The average soil surface temperature was 20 C (maximum 36 C, minimum 15 C). The average air temperature was 21 C (maximum 37 C, minimum 12 C). The initial soil moisture averaged 24% (maximum 28%, minimum 20%), and the ending soil moisture averaged 22 (maximum 25%, minimum 18%) and 19% (maximum 21%, minimum 17%) for the laboratory and field bioassays, respectively.

The results from the field and laboratory bioassays indicated that *S. riobravis* occurred at all six soil depths tested (0–5, 5–10, 10–15, 15–20, 20–25, and 25–30 cm) (Fig. 1). Soil depth differentially affected the occurrence of this nematode, as shown by the field bioassay ($P < 0.002$) and laboratory bioassay ($P < 0.01$) results. The

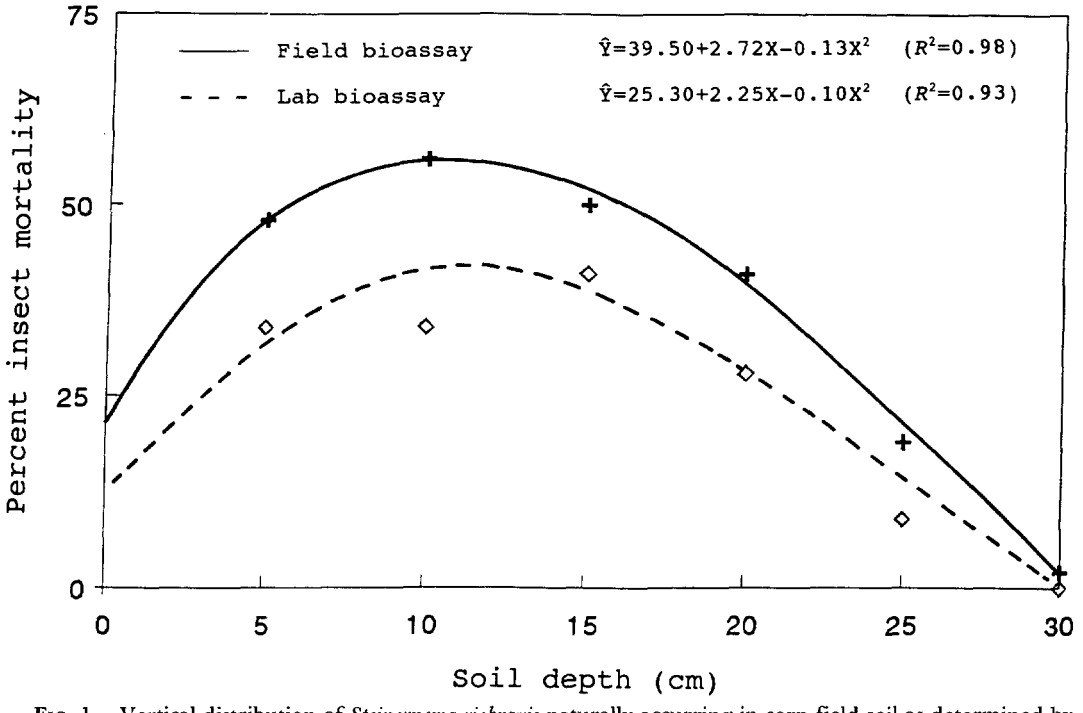

FIG. 1. Vertical distribution of *Steinernema riobravis* naturally occurring in corn field soil as determined by its infectivity on *Helicoverpa zea* from laboratory and field bioassays at 12 days.

highest percentage of parasitism occurred in the upper 20 cm of soil. The lowest percentage of insect mortality due to *S. riobravis* was located at soil depths of 25–30 cm. The field and laboratory bioassays using the top 20 cm soil resulted in *S. riobravis* infection of 49 and 34% of the *H. zea,* respectively, whereas at 25–30 cm soil depths, 11 and 4.5% were infected, respectively.

There were considerable differences in estimating insect mortality between 6 days and 12 days after soil exposure for the field bioassay. For example, the average of insect mortality (prepupae and pupae) was higher when evaluated at 12 days (36%) than at 6 days (6%). The prepupal mortality increased from 4 to 8% when evaluations were performed at 6 days or 12 days, respectively. Similarly, pupal mortality was higher at 12 days (30%) than at 6 days (3%).

A higher percentage of *S. riobravis*-infected pupae resulted from the field bioassay (30%) than from the laboratory bioassay (8%) when insect mortality was compared from both bioassays at 12 days. However, the *S. riobravis* infectivity on prepupae of corn earworm was the same (8%) in both bioassays. The average of insect mortality (over all soil depths), was 36 and 24% for the field and laboratory bioassays, respectively. Averaged over 12 days, the general response of insect mortality (Y) due to *S. riobravis* as a function of soil depth (X) was approximated by a quadratic population density curve (Fig. 1).

The horizontal spatial pattern of *S. riobravis* was aggregated as illustrated in Figure 2. Major differences in nematode distribution resulted in plots separated throughout the field. Averaged over total number of plots, *S. riobravis* occurred in 81, 75, and 31% of the plots corresponding to soil layers of 0–10, 10–20, and 20–30 cm, respectively (Fig. 2). Values for the variance-to-mean ratio were significantly greater than unity (chi-square test) (Table 1). These chi-squared values were well above the upper 5% significance level described by Elliot (7). Therefore, agreement with a Poisson series was rejected at the

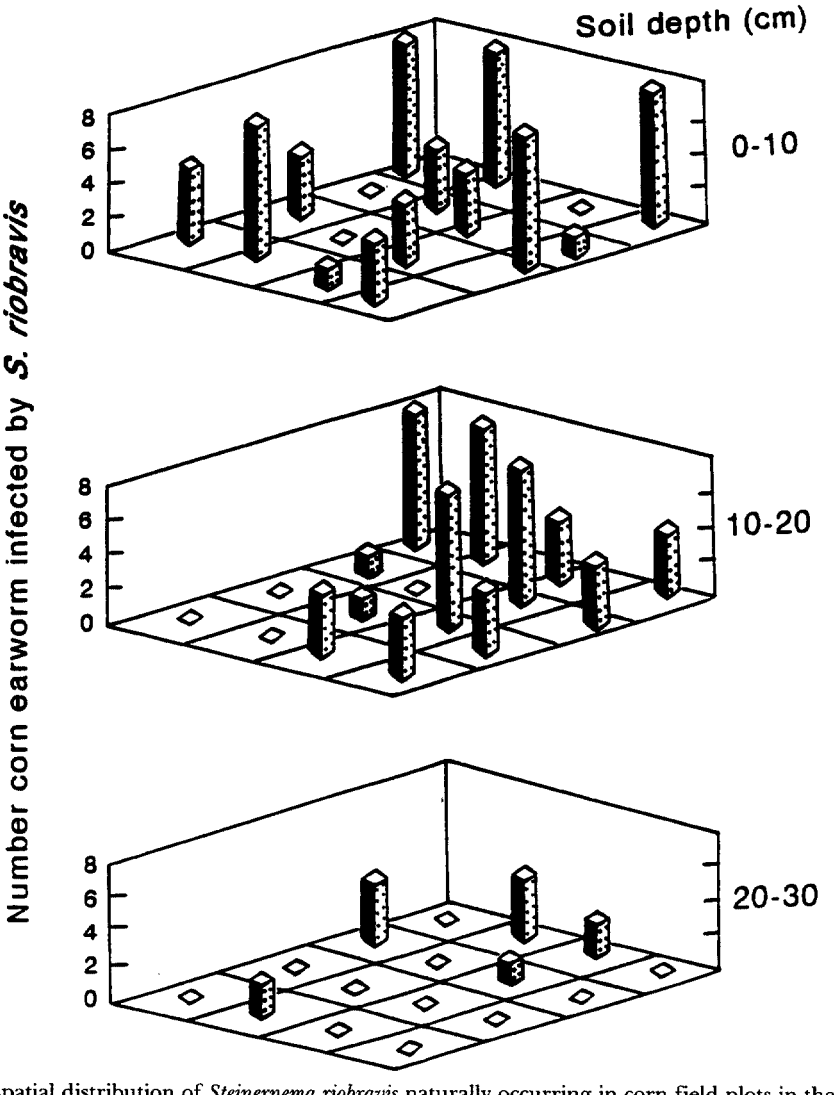

FIG. 2. Spatial distribution of *Steinernema riobravis* naturally occurring in corn field plots in the Lower Rio Grande Valley of Texas. (Numbers are the *S. riobravis*-infected insects, based on eight *Helicoverpa zea* per plot/depth.)

95% probability level ($P < 0.05$). These high values of the variance-to-mean ratio indicated that *S. riobravis* nematodes were aggregated in the plot (Table 1).

DISCUSSION

Although *S. riobravis* occurred throughout the top 30 cm of soil, its horizontal spatial pattern was aggregated. This may explain the uneven and low levels of suppression of corn earworm prepupae and pupae that have been occurring naturally in this area. We observed an average of 11% parasitism (0–20%) of *H. zea* prepupae and pupae caused by *S. riobravis* in this study area (Cabanillas and Raulston, unpubl. data). Plant-parasitic nematodes also have aggregated horizontal spatial distribution, which is most often described by the negative binomial distribution (1). Furthermore, the population of *S. riobravis* is probably influenced by management practices. Generally, row crops predispose the patchiness or nonrandom distribution of nematodes. In contrast, broadcast seeding of plants such as turf and alfalfa may min-

imize the aggregated distribution of nematodes (1). Dispersion indices (k) for this nematode at this particular location with k values of 3.145 in the upper 10-cm soil depth reflects a high degree of clustering (Table 1). This fact should be considered in sampling as well as experimental design for future biocontrol research in this location.

Once the negative binomial distribution has been defined for a population of interest, and the index of dispersion determined, it is possible to calculate the number of samples necessary to estimate the nematode population density with a specified level of precision (1,7).

Corn earworm mortality in our bioassays decreased with soil depth (Fig. 1), suggesting that *S. riobravis* may migrate vertically. Vertical migration could function as an important survival strategy for this nematode. As temperatures change, there may be sufficient time for *S. riobravis* to migrate to greater depths to avoid adverse effects. This may have contributed to the persistence of this nematode even during the absence of corn earworm, which enables it to endure the subtropical environmental conditions of this semi-arid region. In relation to this, we were able to collect viable infective juveniles of *S. riobravis* in November 1991 from fallow field soil where corn had been planted the preceding spring (Cabanillas and Raulston unpubl. data). The normal depth of pupation for corn earworm in soils in the Lower Rio Grande Valley of Texas is about 5 cm around the corn plant (Raulston, pers. comm.), and nematode reproduction probably occurs at this depth. Therefore, decrease in insect bait mortality with depth reflects not the distribution of the insect host but nematode redistribution.

In the Lower Rio Grande Valley, the corn earworm become prepupae in late May and early June, burrow 5–10 cm into the soil around the corn plant, and pupate at a rate of 1–4 pupae/m^2 in commercial corn fields. Nevertheless, the mobility of nematodes and environmental influences may modify this relationship. A characteristic behavior of *S. riobravis* infective juveniles is their high mobility and tendency to move by bridging, leaping, crawling, and climbing (4). Laboratory studies (24 C) on the horizontal movement of *S. riobravis* infective juveniles in the same field soil type (sandy clay loam) showed that they can move an average of 4 cm/day (3–5 cm/day) (Cabanillas and Raulston, unpubl. data). It appears that *S. riobravis* is fairly efficient at horizontal and vertical dispersal in the field.

The laboratory and field bioassays used in this experiment allowed the detection of *S. riobravis* in soil samples, which confirm the results obtained in similar experiments by other researchers (2,14). Although the laboratory bioassay did not show high levels of nematode detection, it was easier than the field bioassay. Our study also shows that consideration should be given to the evaluation time of *Steinernema*-induced insect mortality. The results from the field bioassay indicated that insect mortality was greater when the evaluation was performed at 12 days rather than at 6 days after *H. zea* prepupae were exposed to soil. A delayed evaluation at 12 days allowed the development of nematode prog-

TABLE 1. Statistics, indices of dispersion, and test for agreement with a Poisson series of the spatial distribution of *Steinernema riobravis* at different soil depths in corn plots as determined by field bioassay.

Soil depth (cm)	No. samples† (n)	Mean $\bar{x}$	Variance s^2	$s^2/\bar{x}$	Indices of dispersion	
					k	Chi-square‡
10	16	4.187	9.762	2.33	3.145	34.972
20	16	3.625	9.583	2.64	2.205	39.653
30	16	0.812	2.029	2.50	0.542	37.481

† Each sample consisted of eight pooled subsamples per plot.
‡ For $s^2/\bar{x}$.

eny on dead insects, which provided clear evidence of not only nematode infectivity and pathogenicity on corn earworm but also establishment capacity. This consideration is very important to explain the success or failure in the introduction of biocontrol agents. According to Ehler (6) the numbers of introductions that fail (i.e., no establishment) is greater than those resulting in establishment.

Quantification of entomopathogenic nematode populations in soil for ecological studies is difficult and remains a challenge to be addressed by research workers. At present, the most common procedure used in quantifying nematodes after soil application is using the *Galleria* bioassay technique. Because the number of nematodes invading an insect host is highly variable, no relationship can be drawn between mortality rate and the number of nematodes in the environment (8).

Our study provides new information on the spatial dispersion patterns of *S. riobravis* in its natural habitat and indicates the need to augment its natural biocontrol efficacy. Furthermore, it provides ecological insight about the relative importance of nematode migration in relation to survival in establishing the vertical distribution of *S. riobravis* in the field. This study is significant in that it is the first attempt to quantify the patchiness of entomopathogenic nematodes in the field.

The high level of infectivity of this nematode against corn earworm prepupae as demonstrated in laboratory tests (5), along with its survival in this geographical region, makes *S. riobravis* a potential candidate for biological control.

Literature Cited

1. Barker, K. R., and C. L. Campbell. 1981. Sampling nematode communities. Pp. 3–17 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An advanced treatise on *Meloidogyne,* vol. 2. Methodology. Raleigh: North Carolina State University Graphics.

2. Bedding, R. A., and R. J. Akhurst. 1975. A simple technique for the detection of insect parasitic rhabditid nematodes in soil. Nematologica 21:109–110.

3. Bouyoucos, G. J. 1962. Hydrometer method improved for making particle size analyses of soils. Agronomy Journal 54:464–465.

4. Cabanillas, H. E., G. O. Poinar, Jr., and J. R. Raulston. 1994. *Steinernema riobravis* n. sp. (Rhabditida: Steinernematidae) from Texas. Fundamental and Applied Nematology, in press.

5. Cabanillas, H. E., and J. R. Raulston. 1994. Pathogenicity of *Steinernema riobravis* against corn earworm, *Helicoverpa zea* (Boddie). Fundamental and Applied Nematology, in press.

6. Ehler, L. E. 1990. Some contemporary issues in biological control of insects and their relevance to the use of entomopathogenic nematodes. Pp. 1–19 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

7. Elliot, J. M. 1977. Some methods for statistical analysis of samples of benthic invertebrates. Freshwater Biological Association Scientific Publication No. 25.

8. Georgis, R. 1992. Present and future prospects for entomopathogenic nematode products. Biocontrol Science and Technology 2:83–99.

9. Kaya, H. K. 1990. Soil ecology. Pp. 93–115 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic nematodes in biological control. Boca Raton, FL: CRC Press.

10. Nickle, W. R., J. J. Drea, and J. R. Coulson. 1988. Guidelines for introducing beneficial insect-parasitic nematodes into the United States. Annals of Applied Nematology 2:50–56.

11. Raulston, J. R., S. D. Pair, J. Loera, and H. E. Cabanillas. 1992. Prepupal and pupal parasitism of *Helicoverpa zea* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) by *Steinernema* sp. in cornfields in the Lower Rio Grande Valley. Journal of Economic Entomology 85:1666–1670.

12. Raulston, J. R., S. D. Pair, A. N. Sparks, J. K. Westbrook, J. Loera, K. R. Summy, and D. R. Rummel. 1986. Production of *Heliothis zea* on corn in northeastern Mexico and the Lower Rio Grande Valley of Texas: A potential source for corn and cotton infestation on the High Plains of Texas. Pp. 222–225 *in* Proceedings of 1986 Beltwide Cotton Production Research Conferences. 4–9 January, 1986, Las Vegas, NV. Memphis: National Cotton Council of America.

13. SAS Institute. 1988. SAS/STAT user's guide, release 6.03 ed. Cary, NC: SAS Institute Inc.

14. Saunders, M. C., and J. N. All. 1982. Laboratory extraction methods and field detection of entomophilic rhabditoid nematodes from soil. Environmental Entomology 11:1164–1165.

15. White, C. F. 1927. A method for obtaining infective larvae from culture. Science 66:302–303.